REMARKS

Specification Amendments

The specification has been amended to correct the typographical errors noted by the Examiner. No new subject matter has been added to the claims as a result of these amendments.

Claim Amendments

By the present amendment, claims 3, 6, 8 and 13 have been deleted. Claim 1 has been amended to specify that the method is related to the administration of an effective amount of isolated and purified soyasaponin B_b. Support for this claim amendment is found in the specification on page 4, lines 12-16 and lines 26-28. Composition claims 5 and 7 have been amended to specify that the compositions comprise isolated and purified soyasaponin B_b. Support for these claim amendments is also found in the specification on page 4, lines 12-16 and lines 26-28. Claims 5 and 7 have been amended to clarify that the kidney disease is selected from the group of diseases listed on page 5, lines 11-19, of the specification. Claims 9 and 16-18 have been amended to specify, in part (f), that the sample from (e) is purified by passing the sample through a preparative hydrophobic interaction chromatographic column comprising an electrostatically-linked, aliphatic- or alicyclic-substituted anionic or cationic polysaccharide gel. Support for these amendments is found on page 10, lines 16-19, of the specification and in PCT/CA99/00004 (WO 99/34916), the contents of which were incorporated in the present specification by reference (see page 15, lines 23-26). Claim 14 has been amended to change its dependency from claim 13 to claim 9. Claims 19-20 are new. Claims 19 and 20 are new composition claims dependent on claims 5 and 7, respectively, and specify that the kidney disease is polycystic kidney disease. Support for these claims is found on page 5, lines 19-21, of the specification. The Applicants submit that no new subject matter has been added to the claims as a result of the above amendments.

The amendments to the claims have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicants reserve the right to pursue any of the deleted subject matter in a further divisional, continuation or continuation-in-part application.

The Official Action dated October 25, 2002 has been carefully considered. It is believed that the amendments submitted herewith and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Specification Informalities

The Examiner has objected to page 5 of the specification due to the blank section appearing at the bottom of this page. The Applicants have submitted herewith an amended page 5 wherein the blank section has been crossed out indicating that no material may be added to, or was supposed to appear in, this area. If this is not satisfactory to the Examiner, the Applicants ask the Examiner to indicate how best to correct this typographical error.

The Examiner has further objected to the Tables on pages 16-18 of the specification as some of the entries were not properly aligned. The Applicants have submitted herewith, amended Table pages 16-18 wherein the alignment of the columns has been corrected.

35 U.S.C. §102

The Examiner has rejected claims 1-3 under 35 U.S.C. 102(b) as being anticipated by Philbrick *et al.* (J. Am. Soc. Nephrol., Vol. 10, Sept. 1999, pp 85A, mailed to the subscribers on August 26, 1999, hereinafter Philbrick). The Examiner contends Philbrick discloses the reduction in cyst volumes in mice fed with Group B soyasaponins.

In response, the Applicants have amended claim 1, and thus claim 2, dependent thereon, so that the method of treating kidney disease comprises administering an effective amount of isolated and purified soyasaponin B_b. Philbrick discloses that a soyasaponin-enriched alcohol extract (SEAE) from soy protein isolate (SPI), when fed to mice, was associated with a marked reduction in kidney cyst volumes compared to controls. Philbrick only discloses that the SEAE contains Group B soyasaponins. Philbrick does not teach or suggest that a specific Group B soyasaponin may be responsible for the therapeutic activity of the SEAE. In the present invention it has been found that the specific soyasaponin in the SEAE responsible for the reduction in tumor size in mice is soyasaponin B_b. The present inventors have isolated and purified soyasaponin B_b and added this compound to a diet that was fed to mice and shown it to have the important beneficial effect of intonation of kidney size and cyst development in the *pcy* model of polycystic kidney disease. Philbrick did not identify the active soyasaponin as soyasaponin B_b and did not disclose a method of treating kidney disease comprising administering an effective amount of isolated purified soyasaponin B_b. Accordingly, Philbrick does not anticipate the present invention as claimed in claims 1 and 2. The Applicants have deleted claim 3 without prejudice.

In light of the above, the Applicants respectfully request that the rejection of claims 1-3 under 35 U.S.C. 102(b) be withdrawn.

35 U.S.C. §112, first paragraph

The Examiner has objected to claim 3 under 35 U.S.C. §112, first paragraph as containing subject matter which was not enabled.

Without acquiescing to the Examiner's objections and in order to advance prosecution, the Applicants have deleted claim 3. In view of the foregoing, we respectfully request that the objections to the claims under 35 U.S.C. §112, first paragraph be withdrawn.

35 U.S.C. §112, second paragraph

The Examiner has objected to claims 5 and 7, under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicants regard as the invention. The Examiner contends that the recitation "treating a kidney disease" is indefinite and, accordingly, the claims should be amended to include what disease is being treated.

In response the Applicants have amended claims 5 and 7 so that the kidney disease is selected from the group of kidney diseases listed on page 5, lines 11-19 of the specification.

In view of the foregoing, we respectfully request that the objections to the claims under 35 U.S.C. §112, second paragraph be withdrawn.

35 U.S.C. §103(a)

The Examiner has rejected claims 5-18 under 35 U.S.C. §103(a) as being unpatentable over Collins *et al.* (WO 99/34916) in combination with Collins *et al.* (WO 99/34810), Shinohara *et al.* (USPN 4,217,345) and Miyake *et al.* (USPN 4,557,927). The Examiner contends that it would have been obvious to one of ordinary skill in the art to isolate soyasaponin B_b by using hydrophobic interaction chromatography (HIC) using aqueous acidified ethanol, making pharmaceutical and nutraceutical compositions for treating polycystic kidney disease. We respectfully disagree with the Examiner for the reasons that follow.

The method of isolating soyasaponin B_b described in the present invention did not merely involve HIC using aqueous acidified alcohol. The inventors developed a unique three-step method which was found to work surprisingly well for the isolation of soyasaponin B_b. As described on page 14, line 10, to page 15 line 6, the method involved the main steps of (1) removing polar lipids using HIC on an aliphatic-substituted polysaccharide gel matrix (although other liquid chromatographic

techniques could have been used); (2) anion exchange chromatography in an organic milieu where the soyasaponins are first absorbed onto the column and then eluted off the column using acidified alcohol; and (3) further purification using HIC on "designer gels", such as hexadecyltrimethylammonium-substituted SP Sepharose. The resulting soyasaponin B_b could be further purified using preparative liquid chromatography, recrystallization or other known techniques as desired. It is the unique combination of liquid chromatography, plus anion exchange chromatography using organic solvents and HIC on designer gels that allowed the efficient isolation of soyasaponin B_b from the crude soy processing byproducts described and claimed in the present application. The Applicants submit that the combination of these three steps for the isolation of soyasaponin B_b is not taught nor suggested by either WO 99/349186 or WO 99/34810 for the reasons that follow.

Soybean saponins are complex mixtures containing up to 12 closely related derivatives, and are difficult to individually purify in quantity, even when separated as a group from protein contaminants. WO 99/34810 teaches that a relatively pure mixture of Type B saponins can be isolated from either soy hulls or de-fatted soy flour using hydrophobic interaction chromatography. In the present application, modifications to this technology, which the Applicants submit would not appear obvious to someone skilled in the art of chromatography, have been made to solve specific problems and improve the purification process. Such improvements were necessary since, as stated on page 9, lines 1-20, of the specification, the teachings of WO 99/34810 proved difficult and impractical in implementation, in particular for soy molasses sample sources. The present inventors have found that the combination of hydrophobic interaction chromatography (HIC), in particular the use of so-called designer gels in HIC, combined with ion-exchange in an organic solvent allows a simple and rapid method of overcoming these separation difficulties. Applicants have amended claims 9, and therefore claims 1-12 and 14-15 dependent thereon, as well as claims 16-18, so that it is specified that, in step (f), the sample is purified using liquid chromatography by passing the sample through a preparative hydrophobic

interaction chromatographic column comprising an electrostatically-linked, aliphatic- or alicyclic-substituted anionic or cationic polysaccharide gel.

WO 99/34916 describes the use of so-called designer gels for the isolation and purification of saponins from oat flour and quinoa flour. At neutral pH, these saponins have no charge. The soyasaponins of the present invention are anionic at neutral pH. This significant difference in compound charge would mean their behavior in HIC would be complelely different. Accordingly a person skilled in the art could not predicted that soyasaponin B_b could be separated using HIC and designer gels as described in WO 99/34916.

Finally, WO 99/34916 only describes the use of ion exchange chromatography in aqueous environments for the separation of charged species from the neutral saponins found in oat and quinoa flours. In these applications, the charged species are absorbed onto the column while the neutral saponins are washed through with the eluent. This separation technique would not work with the charged soyasapoins of the present invention. Surprisingly, the introduction of an ion-exchange step, in an organic solvent milieu, possibly allowing for the breakage of ionic association of protein-saponin aggregates (similar to the association aggregates of membrane proteins and surfactants such as SDS, deoxycholate, digitonin, etc, and to the basis of ion-pair chromatographic principles), provided means to overcome impediments to purification of soyasaponin B_b. The micellar nature of saponin-protein complexes, which results in co-chromatography and carry through of impurities with the target saponin, are not well understood and are often difficult to disrupt to allow separation of the individual components. There is no teaching in either WO 99/349186 or WO 99/34810 of the use of anion exchange chromatography using organic solvents for the purification of saponins. The use of organic solvents in anionic exchange chromatography is also relatively rare, adding to the non-obviousness of the method claimed in the present application.

Given the complexity of soy samples and the difficulties involved in isolating individual Group B soyasaponins from these samples, it would not have been obvious to a person skilled in the art, using the teachings of WO 99/349186 or WO 99/34810, to develop the specific three-step procedure for the isolation of soyasaponin B_b claimed in the present application.

Finally, the Applicants submit that a person skilled in the art would not have been led to prepare pharmaceutical or nutraceutical compositions comprising isolated and purified soyasaponin B_b without the knowledge of the polycystic activity of this compound and the means to isolate and purify this compound as taught in the present application. Accordingly, claims 5-8 and 17-18, directed to pharmaceutical compositions comprising isolated and purifed soyasaponin B_b would not have been obvious to a person skilled in the art.

The Applicants wish to point out that claim 16 is dependent on method of treatment claim 1. As outlined above, the Applicants submit that claim 1 is patentable, accordingly, claim 16, dependent thereon, is also patentable.

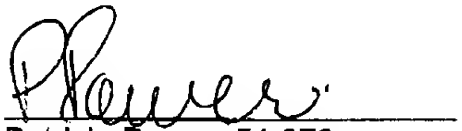
In view of the foregoing, we respectfully request that the objection to the claims under 35 U.S.C. §103(b) be withdrawn.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

In view of the foregoing, we submit that the application is in order for allowance and an early indication to that effect would be greatly appreciated. Should the Examiner

like to discuss the matter, the Examiner is kindly requested to contact Patricia Power at 416-957-1683 at their convenience.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

Please replace specification pages 5 and 16-18 as filed, with amended pages 5 and 16-18 provided herewith.

In the Claims:

Please delete claims 3, 6, 8 and 13.

Please amend claims 1, 5, 9, 14 and 6-18 as follows:

1. (Amended) A method of treating a kidney disease comprising administering an effective amount of [a] isolated and purified soyasaponin B_b to an animal in need thereof.

5. (Amended) A pharmaceutical composition for use in treating a kidney disease comprising an effective amount of [a] isolated and purified soyasaponin B_b in admixture with a suitable diluent or carrier, wherein the kidney disease is selected from the group consisting of polycystic kidney disease, acquired renal cystic disease, medullary cystic disease of the kidney, autosomal recessive polycystic kidney disease, hereditary interstitial nephritis, other inherited disorders in which PKD forms part of the symptomatology, persons with a potassium-wasting disorder, glomerulonephritis and the group of renal disorders associated with inflammatory and immune dysfunction in the kidney.

7. (Amended) A nutraceutical composition for use in treating a kidney disease comprising an effective amount of [a] isolated and purified soyasaponin B_b in admixture with a suitable diluent or carrier, wherein the kidney disease is selected from the group consisting of polycystic kidney disease, acquired renal cystic disease,

medullary cystic disease of the kidney, autosomal recessive polycystic kidney disease, hereditary interstitial nephritis, other inherited disorders in which PKD forms part of the symptomatology, persons with a potassium-wasting disorder, glomerulonephritis and the group of renal disorders associated with inflammatory and immune dysfunction in the kidney.

9. (Amended) A method of isolating soyasaponin B_b from a sample comprising:

(a) solubilizing the sample in acidified aqueous alcohol;

(b) removing polar lipids by liquid chromatography;

(c) solubilizing the sample from (b) in aqueous alcohol;

(d) passing the sample from (c) through an anion exchange column;

(e) eluting the sample absorbed to column in (d) with an acidified aqueous alcohol; and

(f) purifying the sample from (e) by liquid chromatography by passing the sample through a preparative hydrophobic interaction chromatographic column comprising an electrostatically-linked, aliphatic- or alicyclic-substituted anionic or cationic polysaccharide gel and collecting fractions containing soyasaponin B_b.

14. (Amended) A method according to claim [13]9 wherein the preparative hydrophobic interaction column is hexadecyltrimethylammonium-substituted SP Sepharose.

16. (Amended) A method according to claim 1 wherein the soyasaponin B_b is obtained by a method comprising:

(a) solubilizing the sample in acidified aqueous alcohol;

(b) removing polar lipids by liquid chromatography;

(c) solubilizing the sample from (b) in aqueous alcohol;

(d) passing the sample from (c) through an anion exchange column;

(e) eluting the sample absorbed to column in (d) with an acidified aqueous alcohol; and

(f) purifying the sample from (e) by liquid chromatography by passing the sample through a preparative hydrophobic interaction chromatographic column comprising an electrostatically-linked, aliphatic- or alicyclic-substituted anionic or cationic polysaccharide gel and collecting fractions containing soyasaponin B_b.

17. (Amended) A pharmaceutical composition according to claim 5 wherein the soyasaponin B_b is obtained by a method comprising:

(a) solubilizing the sample in acidified aqueous alcohol;

(b) removing polar lipids by liquid chromatography;

(c) solubilizing the sample from (b) in aqueous alcohol;

(d) passing the sample from (c) through an anion exchange column;

(e) eluting the sample absorbed to column in (d) with an acidified aqueous alcohol; and

(f) purifying the sample from (e) by liquid chromatography by passing the sample through a preparative hydrophobic interaction chromatographic column comprising an electrostatically-linked, aliphatic- or alicyclic-substituted anionic or cationic polysaccharide gel and collecting fractions containing soyasaponin B_b.

18. (Amended) A nutraceutical composition according to claim 7 wherein the soyasaponin B_b is obtained by a method comprising:

(a) solubilizing the sample in acidified aqueous alcohol;

(b) removing polar lipids by liquid chromatography;

(c) solubilizing the sample from (b) in aqueous alcohol;

(d) passing the sample from (c) through an anion exchange column;

(e) eluting the sample absorbed to column in (d) with an acidified aqueous alcohol; and

(f) purifying the sample from (e) by liquid chromatography by passing the sample through a preparative hydrophobic interaction chromatographic column comprising an electrostatically-linked, aliphatic- or alicyclic-substituted anionic or cationic polysaccharide gel and collecting fractions containing soyasaponin B_b.

Please add the following new claims:

19. (New) The composition according to claim 5, wherein the kidney disease is polycystic kidney disease.

20. (New) The composition according to claim 7, wherein the kidney disease is polycystic kidney disease.

disease. Examples of analogs of soyasaponinB_b include compounds sharing the structural backbone of soyasaponin B_b as shown in Figure 3 including soyasaponin B_a (Figure 4) or soyasaponin B_c (Figure 5). (Shiraiwa et a., Agri. Biol. Chem., 55(4):911-917, 1991).

The term "effective amount" as used herein means an amount effective, at dosages and for periods of time necessary to achieve the desired results.

The term "animal" as used herein includes all members of the animal kingdom, including humans. Preferably, the animal to be treated is a human.

The term "kidney disease" as used herein means any condition that affects kidney or renal function including polycystic kidney disease, acquired renal cystic disease, medullary cystic disease of the kidney, autosomal recessive polycystic kidney disease, hereditary interstitial nephritis, other inherited disorders in which PKD forms part of the symptomatology (e.g. Oral Facial Digital Syndrome), persons with a potassium-wasting disorder (Hypokalemia which also leads to renal cystic formation), glomerulonephritis and the group of renal disorders associated with inflammatory and immune disfunction in the kidney. Preferably, the kidney disease treated according to the present invention is polycystic kidney disease.

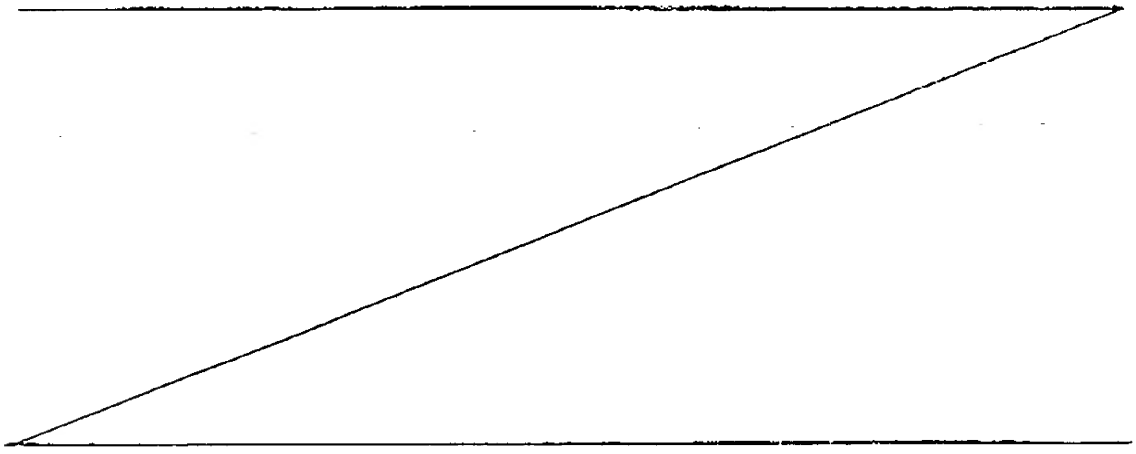

TABLE 1

Diet Composition

Ingredient	Casein	Casein+ Novasoy 400®	Casein+ Soyasaponin B_b
	(Weight % of the Total Diet)		
Casein protein	15	15	15
Vitamins +amino acids	1.2	1.2	1.2
Salts + salt supplement	5.5	5.5	5.5
Corn oil	15	15	15
Antioxidant	0.05	0.05	0.05
Sucrose + cornstarch	60.8	60.8	60.8
Fibre	2.45	0.5	2.3
Novasoy 400®	----	2	---
Bb concentrate	---	---	0.18
Totals	100	100.1	100

Note about these diets:

1) The amount of the soyasaponin B_b concentrate was based on the analyses of a saponin-rich alcohol extract (SEAE) used in a previous experiment. This crude extract was found to contain 181 mg soyasaponin B_b/mg extract; addition of the purified soyasaponin B_b concentrate at a level of 0.18% of the total diet would provide a similar dietary intake provided by the SEAE.
2) The Novasoy 400® was used as the source of the purified soyasaponin B_b concentrate and was added to the casein-based diet at a level (2% of the total diet) which would give equivalent amounts of the Group B soyasaponins as that provided by the concentrate.
3) The three diets contained the same amounts of food energy and protein.

TABLE 2

Body and tissue weight in *pcy* mice fed a unsupplemented casein-based diet or this diet supplemented with Novasoy 400® or the soyasaponin B_b concentrate

Measurement	Treatment		
	Casein	Casein+ Novasoy 400®	Casein + Soyasaponin B_b
Total kidney wt (g)	1.8±0.1	1.3±0.1*	1.3±0.1*
Total kidney wt (g/100 g body wt)	7.0±0.3	5.4±0.3*	5.2±0.3*
Kidney water content (g/left kidney)	0.82±0.1	0.63±0.1*	0.61±0.1*
Final body wt (g)	25.2±0.9	23.3±0.8	25.1±0.1
Liver wt (g)	1.2±0.1	1.3±0.1	1.2±0.2
Liver wt (g/100 g body wt)	4.6±0.1	5.6±0.4*	4.6±0.1
Liver water content (g)	0.84±0.0	0.95±0.1	0.82±0.0

Data are averages ± standard error of the means for 8 animals/ group. Values marked by asterisks (*) are significantly different ($p<0.05$) from the casein-fed mice.

TABLE 3

Clinical Chemistries from *pcy* mice fed the unsupplemented casein diet or this diet supplemented with either Novasoy 400® or the soyasaponin Bb concentrate

Measurement	Treatment		
	Casein	Casein + Novasoy 400®	Casein + soyasaponin B_b concentrate
Plasma creatinine (mM/L)	30.9±9.1	19.0±2.5*	17.5±1.9*
Plasma cholesterol (mM/L)	5.3±0.2	4.3±0.2*	4.8±0.3*
Plasma urea (mM/L)	22.1±2.3	15.8±3.2	19.5±3.0
Plasma total protein (g/L)	56.0±1.9	55.7±0.8	54.3±1.1

Average ± standard error of the means are shown for 8 animals/group. Values marked by asterisks (*) are significantly different ($p<0.05$) from the casein-fed mice.